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In the Specification

Amend paragraph nos. 0008, 0012, 0014, 0016, 0022, 0026, 0027, and 0042, as follows:

[0008] The present invention relates to a method for inhibiting proteolysis of silage by pretreating the forage with a plant-derived polyphenol oxidase (PPO) in conjunction with an ~~o-diphenyl~~ o-diphenol compound. More specifically we have found that red clover polyphenol oxidase or other plant derived polyphenol oxidase (PPO) in conjunction with an ~~o-diphenyl~~ o-diphenol compound prevents excessive pyrolysis of proteins. The inventive process includes the transformation of forages such as alfalfa to produce PPO that would simply require the addition of an ~~o-diphenyl, compound~~ o-diphenol compound such as caffeic acid, or the use of PPO transformed plants as a source of PPO to be added to other forages to be ensiled.

[0012] The present invention involves the creation of ensiled forages that are resistant to excessive proteolysis. This is accomplished by the treating of a plant material to be ensiled with combinations of polyphenol oxidase (PPO) and an o-diphenol compound such as catechol or caffeic acid and its derivatives (e.g. chlorogenic acid, caffeoyl tartrates, caffeoyl glucose,

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phasic acid, clovamide and rosmarinic acid). In most forages, PPO activity is too low for the generation of sufficient free quinones to efficiently inactivate proteolytic enzymes. However, proteolysis can be inhibited in any forage through application of PPO and ~~a~~ an o-diphenol compound.

[0014] The ~~o-diphenyl~~ o-diphenol compound is applied to forage at a rate ranging from about 5 to about 30 micromoles per gram of fresh weight forage and the oxidase is applied at a rate of about 0.1 to about 1 unit per gram of fresh weight forage. One unit is defined as one micromole of caffeic acid oxidized per minute by the following protocol.

[0016] The PPO can be added as a water soluble solution. The ~~o-diphenyl~~ o-diphenol may be added as a water/ethanol solution to ensure good solubility. The determination of an optimal water/ethanol mix would be determinable by one of ordinary skill in the field.

[0022] Inhibition of proteolysis in any ensiled forage is dependent upon the processing of the forage by grinding, chopping, and/or severely conditioning and exposing it at that

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time to ~~o-diphenyl~~ o-diphenol compounds and PPO. Since PPO generated o-quinones must come in contact with proteases to shut them down, application of PPO and ~~o-diphenyl~~ o-diphenol compound must occur at the time of chopping of the forage. The greater the degree of chopping/maceration of the forage the more effective the process will be for proteolytic inhibition. The reaction of PPO to produce o-quinones is oxygen dependent and therefore becomes self regulated in the silage process. The degree of chopping/maceration for effective functioning of this invention is from 30 to 60 Conditioning Index (CI), with a preferred range from about 45 to 55 Conditioning Index(CI).

Conditioning Index (CI) is herein defined as the

$$CI = 100 \times \frac{\text{Forage conditioned leachate conductivity}}{\text{Waring Blender leachate conductivity}}$$

[0026] The term o-diphenol is herein defined as those o-diphenols that can effectively react with polyphenol oxidases and may include catechol and caffeic acid and related compounds. These compounds are herein defined as containing two hydroxyl groups in an ortho position on a phenyl ring. Substitutions could be as in caffeic acid, ~~phenyl~~ an o-diphenol with a three carbon chain or its derivatives, chlorogenic acid, clovamide or

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caffeoyl malates. Although any o-diphenol should work, we have found that caffeic acid or its derivatives and catechol and related compounds are the best substrates for this PPO reaction. An effective amount of o-diphenol is defined herein as the amount needed to achieve at least 20% proteolytic inhibition.

[0027] The term "effective amount" is used herein to refer to the quantity of ~~o-diphenyl~~ o-diphenol compound and polyphenol oxidase necessary to achieve a reduction in proteolysis of ensiled material as compared to an untreated control under suitable conditions of treatment as defined herein.

[0042] The results of the examples suggest there may be a low level of PPO and o-diphenol activity already within the forage but both it and the level of natural phenols is too low to result in adequate proteolytic inhibition. Through the addition of both PPO and ~~o-diphenyl~~ o-diphenol compounds, it is possible to control the level of o-quinones produced in order to optimize proteolytic inhibition without excessive reaction that has the potential for decreasing the nutritional value of the native proteins. PPO can be added as a water soluble solution. The o-diphenols are optimally added as a water/ethanol solution to ensure good solubility.